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ABSTRACT: The objective of this experiment was to determine the effect of different doses of vitamin D_3 (VITD) on beef feedlot performance, plasma and muscle Ca^{2+}, tissue residues, and improvement of Warner-Bratzler shear force (WBS) and panel tenderness. A total of 167 steers were fed one of six levels of VITD. The VITD treatments (28 steers/treatment) were 0, 0.5×10^6, 1×10^6, 2.5×10^6, 5×10^6, and 7.5×10^6 IU/steer daily of VITD fed nine consecutive days before slaughter. Feedlot performance and plasma Ca^{2+} were measured during the last 21 days on feed. Warner-Bratzler shear force was measured on strip loin and top round steaks at 7, 10, 14, and 21 d postmortem. The VITD treatments of 5 and 7.5×10^6 IU/steer daily decreased ($P < 0.05$) ADG, and VITD supplementation of 2.5, 5, and 7.5×10^6 IU/steer daily decreased average dry matter feed intake ($P < 0.05$) at the end of the feeding trial. Plasma Ca^{2+} increased linearly with VITD treatment ($P < 0.01$). Calpastatin and calpain activity were not influenced by treatment ($P > 0.05$), but muscle Ca^{2+} was increased ($P < 0.05$) by VITD treatments of 1, 2.5, 5, and 7.5 10^6 IU/steer daily. Feeding VITD did not influence ($P > 0.05$) carcass quality or yield traits. Supplementing VITD at levels of 1, 2.5, 5, and 7.5 10^6 IU/steer daily increased ($P < 0.05$) VITD concentrations in strip loin and liver samples. Cooking liver decreased VITD concentrations 10 to 28%. Vitamin D_3 treatments of 0.5 and 7.5×10^6 IU/d reduced strip loin steak WBS at d 7 ($P < 0.05$), but VITD treatments did not decrease strip loin steak WBS at any other time postmortem. The VITD treatments of 0.5, 1, and 5×10^6 IU/steer daily decreased top round steak WBS at 7 d, and all VITD treatments decreased 10-d top round steak WBS ($P < 0.05$). Supplementing steers with 0.5×10^6 IU/steer daily of VITD also decreased ($P < 0.05$) top round steak WBS at 21 d postmortem compared with controls. Sensory tenderness at 7 d postmortem was increased ($P < 0.05$) by all VITD treatments in top round steaks, yet strip loin tenderness scores were not affected ($P > 0.05$) by VITD treatment. Treatment with VITD quadratically decreased ($P < 0.05$) round WBS. Thus, VITD treatment will effectively improve tenderness when cattle tend to be tough and have no impact on cattle that produce tender beef. Feeding steers 0.5×10^6 IU of VITD daily for 9 d improved tenderness in two muscles without negatively affecting feedlot performance or tissue residues.

Key Words: Beef, Calcium, Residues, Tenderness, Vitamin D

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Introduction

Tenderness is the most variable and single most important factor affecting consumer satisfaction with meat (Savell et al., 1987; Smith et al., 1987; Miller et al., 1998). Surveys of retailers and restaurateurs indicated beef tenderness varies greatly by and within muscles (Morgan et al., 1991; Hamby, 1992; Brooks et al., 2000). Inadequate tenderness has been estimated to cost the U. S. beef industry $200 to $300 million annually (Smith et al., 1995; Morgan, 1995; Miller et al., 1998). Myofibrillar proteolysis from the intracellular calcium-dependent proteases, μ-calpain and m-calpain, has enhanced meat tenderization (Koohmaraie, 1992; Huff-Lonergan et al., 1996a). Thus, increasing muscle calcium antemortem may activate calpains and improve beef tenderness.

Vitamin D_3 (**VITD**) plays a vital role in maintaining blood concentrations of calcium (Horst, 1986; Hurwitz, 1996). Early studies with VITD indicated that as low as 1×10^6 IU/d increased blood calcium and decreased

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the incidence of milk fever in dairy cows (Hibbs et al., 1946, 1951; Hibbs and Pounden, 1955). Injections of the VITD metabolites 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 also have been shown to increase serum calcium concentrations (Hollis et al., 1977; Hove et al., 1983; Hodnett et al., 1992).

Swanek et al. (1999) and Montgomery et al. (2000) reported VITD supplementation to steers at 5×10^6 and 7.5×10^6 IU/d improved beef longissimus tenderness. Thus, VITD supplementation could act as other calcium-induced systems such as calcium chloride injection and infusion (Koohmarie et al., 1988; Kerth et al., 1995). Before vitamin D supplementation can be implemented by the beef industry to improve tenderness, the dose of VITD requires titration. Issues of potential residues and negative animal performance also require characterization. The objectives of our study were to determine the effects of the dose of vitamin D_3 on beef tenderness, residues, and feedlot performance.

Materials and Methods

Design of the Experiment

Crossbred steers were supplemented with different doses of VITD to determine the effects of supplementation on beef tenderness. A total of 168 steers were weighed and separated into seven weight groups. From each of the weight groups, steers were assigned randomly to six different pens of four steers each. All steers were fed a typical Texas High Plains high-concentrate finishing diet with ad libitum access. The diet consisted of (as-fed basis) 76.75% whole steam-flaked milo, 8.02% cottonseed hulls, 3.37% whole steam-flaked corn, 3.25% cane molasses, 2.27% animal fat, 1.88% cottonseed meal, 0.49% urea, and 3.97% supplement (24.92% calcium carbonate, 22.72% Rumensin [Elanco Animal Health, Greenfield, IN], 13.76% Tylosin [Elanco], 12.40% vitamin E [Roche Vitamins, Nutley, NJ], 9.81% potassium chloride, 7.52% vitamin A [Roche], 5.77% trace minerals, and 3.10% salt). Before steers received vitamin D_3 treatment, one steer was removed from the experiment because of a leg injury, leaving 167 steers remaining in the experiment. The last 9 d of the feeding period the steers were supplemented with 0, 0.5, 1, 2.5, 5, or 7.5×10^6 IU of VITD/steer daily (Roche). Supplemental VITD was added to the high-concentrate finishing diet and thoroughly mixed, and feed intake was measured daily per pen during supplementation. Blood plasma samples were collected for ionized Ca^{2+} analysis 21, 8, and 3 d before slaughter and at slaughter during exsanguination. Concentrations of plasma Ca^{2+} were determined in duplicate by atomic absorption spectrometry (Perkin-Elmer Corp., 1965) using standards of 5, 10, and 15 mg of Ca^{2+}/100 mL on a Perkin Elmer model 2380 atomic absorption spectrometer (Perkin Elmer, Wellesley, MA).

After the 9 d of VITD supplementation, the steers were transported the following morning to a USDA inspected facility and slaughtered using approved humane techniques. Liver samples were collected from the right hepatic lobe (lobus hepatis dexter), and the remaining liver was discarded if the steer had been supplemented with VITD. A 30-g longissimus muscle sample was removed from one steer per pen (n = 42) at 20 min postmortem for calpastatin and calpain determination according to procedures of Koohmaraie (1990). Carcass temperature and pH were measured between the 11th and 12th ribs in the longissimus at 3 and 12 h postmortem using a Hantover Model TM99A-H digital thermometer (Hantover, Middlefield, CT), and carcass pH was measured with a Model 230A Orion temperature-compensated pH meter (Orion Research, Boston, MA).

Following a 48-h chilling period (–1°C), the carcasses were ribbed, and USDA quality and yield traits were recorded. Commission Internationale de l'Eclairage (CIE) L* (muscle lightness), a* (muscle redness), b* (muscle yellowness), saturation index, and hue angle values were collected from two random readings on each carcass ribeye with a Minolta spectrophotometer (Model CM-2002, Minolta Corp., Ramsey, NJ) using illuminant D_{65} and a 1-cm aperture. After collection of USDA grade and color information, one strip loin (IMPS # 180) and one top round (IMPS # 168) were collected from the left side of each carcass (USDA, 1990). Strip loin and top round steaks were cut 2.54 cm thick, placed in Cryovac B160 beef bags, and wet-aged at 2°C. Strip loin and top round steaks were aged to 7, 10, 14, or 21 d for Warner-Bratzler shear (**WBS**) force determinations and to 7 d postmortem for sensory evaluations, as well as for chemical and water analysis. At each of the individual aging treatments, steaks were frozen at –20°C until subsequent analyses.

Water-Holding Capacity and Chemical Analysis of Fresh Beef

Percentage of moisture and of free, bound, and immobilized water were determined on longissimus samples using the procedure of Wierbicki and Deatherage (1958). Muscle calcium and phosphorus concentrations also were determined on longissimus samples according to AOAC (1990) guidelines. A 5-g muscle sample was placed in a crucible and dried in a vacuum drying oven for 12 h. Samples were then ashed in a muffle furnace at 600°C for 18 h. Samples were then cooled to room temperature and disolved in 15 mL of 20% (vol/vol) HCl. Samples were then filtered into a 100-mL flask and brought to volume with distilled water. Next, 4 mL of the diluted sample was placed in a test tube with 6 mL of distilled water and 0.5 mL of 5% (vol/vol) lanthanum chloride solution. Calcium concentration was determined in duplicate using atomic absorption as described previously. Phosphorus was determined in duplicate according to AOAC (1990) procedures colorimetrically using a Beckman DU-50 spectrophotometer (Beckman Coulter, Chaska, MN). The spectrophotome-

ter was calibrated before use with P standards of 0, 1, 3, 6, and 9 mg of P/100 mL of solution.

Vitamin D_3, 25-hydroxyvitamin D_3, and 1,25-dihydroxyvitamin D_3 in Beef and Liver

Vitamin D_3, 25-hydroxyvitamin D_3, and 1,25-dihydroxyvitamin D_3 were quantified by a modification of the methods of Montgomery et al. (2000). Briefly, 8 mL of phosphate-buffered saline was placed in a test tube containing 2 g of thinly sliced tissue and homogenized with a Polytron (Kinematica AG, Littan-Lucerne, Switzerland). A 2-mL aliquot of the homogenate (0.4 g of tissue) was transferred to a 25- × 100-mm glass centrifuge tube. Approximately 50 ng of vitamin D_2, 1,000 cpm of [^{3}H]25-hydroxyvitamin D_3, and 1,000 cpm of [^{3}H]1,25-dihydroxyvitamin D_3 were added to the 2-mL aliquot samples for recovery estimates. Vitamin D_3, 25-hydroxyvitamin D_3, and 1,25-dihydroxyvitamin D_3 were then determined in fresh longissimus and liver samples according to Montgomery et al. (2000). For cooked liver samples, the 2-mL aliquot of the homogenate (0.4 g of tissue) was placed in 25- × 100-mm glass tubes then heated in a hot water bath to 75°C for 5 min to simulate cooking. Samples were then cooled to room temperature and capped, and vitamin D_3, 25-hydroxyvitamin D_3, and 1,25-dihydroxyvitamin D_3 were determined according to Montgomery et al. (2000). Recovery estimates averaged approximately 85, 65, and 50% for VITD, 25-hydroxyvitamin D_3, and 1,25-dihydroxyvitamin D_3, respectively.

Tenderness Determination

Steaks for WBS and sensory determinations were thawed in a 2°C cooler for 24 h and then cooked on a gas grill (model 6124RCB, Star Mfg., Smithville, TN) with a grill-surface cooking temperature of 181°C. The beginning internal steak temperature before grilling was 3°C ± 1. Steaks were turned at 3-min intervals to prevent charring, until an internal temperature of 71°C was achieved. Warner-Bratzler shear force determinations and trained sensory panel evaluations for tenderness were made according to AMSA (1995). Steaks for WBS evaluation were placed on plastic trays, covered with polyvinyl chloride film, and chilled for 18 h at 2°C. Six cores (1.27 cm in diameter) were removed from each steak parallel to the muscle fiber orientation and sheared once with a WBS machine (G-R Elec. Mfg., Manhattan, KS). The shear force determination for the six different cores within a steak were then averaged. Sensory steaks were cut hot into 1-cm^3 cubes immediately after cooking and stored in warming pans (approximately 5 min) until they were served warm (approximately 50°C) to the sensory panel. Samples were evaluated by an eight-member sensory panel trained according to Cross et al. (1978). Steaks were evaluated for initial juiciness, sustained juiciness, initial tenderness, sustained tenderness, flavor intensity, beef flavor, overall mouth feel (8 = extremely juicy, tender, intense, characteristic beef flavor, beef-like mouth feel to 1 = extremely dry, tough, bland, uncharacteristic beef flavor, non-beef-like mouth feel), and off-flavor (5 = extremely off-flavor to 1 = none).

Statistical Analyses

For average feedlot performance, carcass traits, plasma and muscle calcium and phosphorus values, water-holding capacity, and tissue residues, data were analyzed as a completely randomized block design that tested the main effect of VITD treatment and weight block using pen as the experimental unit. For sensory characteristics, data were analyzed as a completely randomized block design with a split-plot arrangement using a model that tested the main effect of VITD treatment, with muscle type and all interactions represented in the subplot. For WBS, data were analyzed as a completely randomized block design with a split-split plot arrangement. The main effects and first subplot were as above, and the second subplot tested effects of postmortem aging periods and all interactions. For average daily feed intake during the supplementation period, data were analyzed as a completely randomized block design with a split-plot arrangement using a model that tested the main effect of VITD treatment and day, and all interactions were represented in the subplot. Pen was the experimental unit and an alpha level of 5% was used. Least squares means were calculated using Proc GLM procedures of SAS (SAS Inst. Inc., Cary, NC), and treatment differences were determined using the PDIFF option. Linear and quadratic relationships of VITD dose also were tested using Proc GLM procedures.

Results

Vitamin D_3 supplementation effects on feedlot performance during the last 21 d of the feeding period are presented in Table 1. Vitamin D_3 supplementation had no effect on initial body weight, final weight, or average daily feed intake ($P > 0.05$). Vitamin D_3 treatment linearly decreased ($P < 0.01$) average daily gain across the last 21 d of feeding with VITD supplementation rates of 5 and 7.5×10^6 IU/steer daily, resulting in negative average daily gains that differed from those of steers treated with 1×10^6 IU/steer daily or less ($P = 0.02$; Tables 1 and 2). Although average daily feed intake across the last 21 d of the feeding period was not affected by VITD supplementation (Table 1), there was a VITD × day interaction ($P < 0.002$; Figure 1) when feed intake was measured during the 9-d supplementation period. Vitamin D_3 supplementation resulted in a linear decrease ($P < 0.01$) in feed intake during the last 3 d of supplementation. Specifically, supplementing steers with 2.5, 5, or 7.5×10^6 IU/steer daily of VITD decreased feed intake during d 7 and 8 compared with that of control steers ($P < 0.05$; Figure 1), whereas none of the

Table 1. Effect of vitamin D_3 supplementation to steers for nine consecutive days before slaughter on initial and final weights, feed intake, and average daily gain during the last 21 d of feeding

	Vitamin D_3 treatment, $\times 10^6$ IU/steer daily							
Item	Control	0.5	1.0	2.5	5.0	7.5	*P*-value	SEM
Initial weight before treatment, kg	504	502	506	507	503	506	0.986	5
Final weight, kg	511	511	509	506	500	495	0.580	7
Average daily feed intake during treatment, kg of DM/steer	5.47	5.39	5.17	4.72	4.58	4.26	0.382	0.22
Average daily gain, kg	0.35[a]	0.43[a]	0.21[a]	–0.04[ab]	–0.16[b]	–0.54[b]	0.021	0.20

[a,b]Means in the same row with different superscripts differ ($P < 0.05$).

VITD treatments affected ($P > 0.05$) feed intake during the first 6 d of supplementation. Supplementing steers with 0.5 or 1×10^6 IU/steer daily of vitamin D_3 for nine consecutive days did not negatively affect average daily gain or feed intake.

Plasma calcium was not affected ($P > 0.05$) before supplementation or at d 2 of VITD supplementation (Figure 2). All VITD treatments tested increased ($P < 0.001$) plasma calcium concentrations compared to controls at d 7 of supplementation and at slaughter. Increasing VITD dose linearly increased plasma Ca^{2+} concentrations on d 7 ($P < 0.05$) of supplementation and at slaughter ($P < 0.01$), resulting in as much as a 20% increase of plasma calcium.

Vitamin D_3 supplementation effects on carcass yield, quality, and color traits are shown in Table 3. Vitamin D_3 supplementation did not affect ($P > 0.05$) any of the USDA yield or quality traits or subjective lean color, firmness, or texture scores ($P > 0.05$). Although VITD treatments had some effects on average daily gain and

Table 2. Linear contrasts for feedlot performance, carcass traits, muscle and plasma calcium concentration, tissue vitamin D residues, and Warner-Bratzler shear force from steers supplemented vitamin D_3 for nine consecutive days before slaughter

	Significant contrasts		
Variable	Overall vitamin D_3 treatment	Linear treatment effect	Quadratic treatment effect
Feed intake d 7	*	**	NS
Feed intake d 8	NS	**	NS
Feed intake d 9	*	**	NS
Overall average feed intake	**	**	NS
Average daily gain	**	**	NS
Meat calcium concentration	**	**	NS
Plasma calcium at slaughter	**	**	NS
CIE L* values	NS	NS	NS
CIE a* values	*	NS	*
CIE b* values	**	NS	**
Saturation index	*	NS	*
3-h Carcass temperature	**	**	NS
24-h Carcass temperature	**	**	**
3-h Carcass pH	NS	NS	NS
24-h Carcass pH	**	NS	*
Vitamin D_3 concentration in beef	**	**	NS
Vitamin D_3 concentration in liver	**	**	NS
25-Hydroxyvitamin D_3 in liver	**	**	NS
25-Hydroxyvitamin D_3 in beef	**	**	NS
1,25-Dihydroxyvitamin D_3 liver	*	**	NS
7-d Strip loin steak WBS	**	NS	NS
10-d Strip loin steak WBS	NS	NS	NS
14-d Strip loin steak WBS	NS	NS	NS
21-d Strip loin steak WBS	NS	NS	NS
7-d Top round steak WBS	**	NS	**
10-d Top round steak WBS	**	**	*
14-d Top round steak WBS	NS	NS	NS
21-d Top round steak WBS	NS	NS	NS

[a]NS = not significant ($P > 0.05$).
*$P < 0.05$.
**$P < 0.01$.

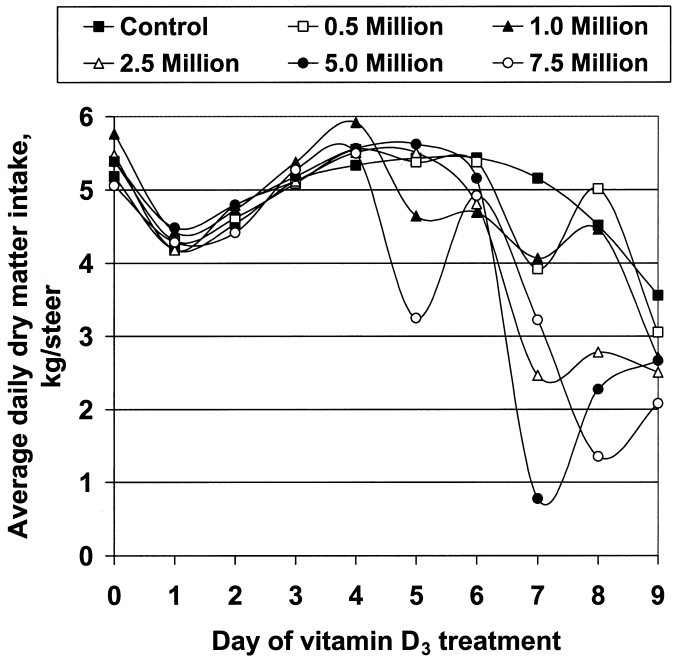

Figure 1. Influence of feeding vitamin D_3 to steers for nine consecutive days before slaughter on average daily dry matter intake. The last day of treatment and feeding was d 9, and steers were slaughtered the following day. The standard error was 0.48; the vitamin D_3 treatment × day interaction *P*-value was 0.002.

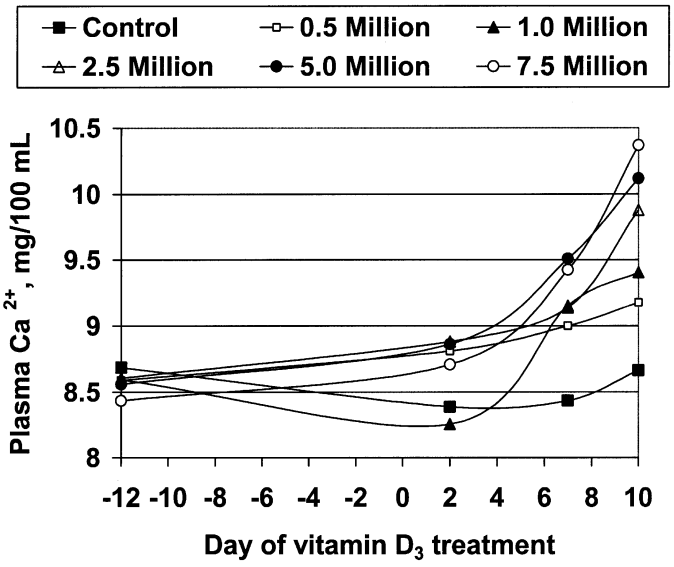

Figure 2. Influence of feeding vitamin D_3 to steers for nine consecutive days before slaughter on plasma calcium concentration. Steers were slaughtered on d 10. Vitamin D_3 treatment *P*-values were 0.841, 0.360, 0.003, and < 0.001 for the individual days, respectively. Standard error of the means were 0.12, 0.39, 0.30, and 0.11 for the individual days, respectively.

daily feed intake, it is important to note that hot carcass weight and dressing percentage were not affected ($P > 0.05$) by VITD supplementation for 9 d. Therefore, improvements in beef tenderness resulting from vitamin D_3 supplementation could be made without negatively affecting economically important carcass factors. Supplementing steers with 0.5 or 1×10^6 IU/steer daily of VITD resulted in lower ($P = 0.009$) carcass ribeye CIE b* values (lean yellowness) compared with controls. Vitamin D_3 treatment quadratically decreased CIE a* (lean redness) and b* values and saturation indexes ($P < 0.05$), whereas CIE L* values and hue angle were not affected ($P > 0.05$; Tables 2 and 3). Thus, using objective lean color measurements (CIE a* and b* values) to classify carcasses as tough or tender, such as in Wulf et al. (1997), would possibly have decreased effectiveness when carcasses are from VITD-fed cattle.

Muscle calcium was increased ($P < 0.008$) by VITD treatments of 1×10^6 IU/steer daily and greater than that in controls (Table 4). Although muscle calcium was linearly increased by treatment ($P < 0.01$), muscle phosphorus content was not affected ($P > 0.05$). Percentages of muscle moisture, free water, bound water, and immobilized water were not affected ($P > 0.05$) by VITD treatment. Longissimus μ- and m-calpain and calpastatin activities also were not affected by VITD treatment ($P > 0.05$). Carcass temperature measured at 3 h postmortem was linearly decreased with increasing VITD dose ($P < 0.01$; Table 2). There also was a linear decrease ($P < 0.01$) in 24-h carcass temperature when steers had been treated with 1×10^6 IU/steer daily of VITD or greater. Carcass pH at 3 h postmortem was not affected by treatment, whereas 24-h carcass pH was quadratically increased ($P < 0.05$) by VITD treatment (Table 4). Vitamin D_3 treatment effects on pH and temperature could possibly be attributed to increased muscle calcium and increased muscle metabolism in response to increased concentrations of calcium.

The effects of supplementation on concentrations of vitamin D_3, 25-hydroxyvitamin D_3, and 1,25-dihydroxyvitamin D_3 in strip loin beef and liver samples are presented in Table 5. Supplementing steers with levels of 1×10^6 IU/steer daily of VITD and greater for nine consecutive days increased vitamin D_3 concentrations in beef and uncooked liver samples ($P < 0.001$). Treating steers with VITD levels of 5 or 7.5×10^6 IU/steer daily increased ($P < 0.05$) 25-hydroxyvitamin D_3 concentrations in beef and fresh liver samples compared with controls. Supplementing the high dose resulted in vitamin D_3 concentrations 6.7 times and 3.6 times higher than those in control strip loin and liver samples, respectively. Increasing VITD dose linearly increased ($P < 0.01$) vitamin D_3 and 25-hydroxyvitamin D_3 concentrations in the raw beef and liver samples. Vitamin D_3 concentrations were increased ($P = 0.01$) in cooked liver samples when steers were supplemented with VITD levels of 2.5×10^6 IU/steer daily and higher. Cooked liver 25-hydroxyvitamin D_3 concentrations also were increased ($P = 0.003$) by supplementing steers with 5

Table 3. Effect of feeding vitamin D_3 to steers for nine consecutive days before slaughter on carcass yield and quality grading factors and color traits

Item	Vitamin D_3 treatment, $\times 10^6$ IU/steer daily							
	Control	0.5	1.0	2.5	5.0	7.5	*P*-value	SEM
Hot carcass weight, kg	322.2	317.6	318.5	316.0	314.8	315.3	0.909	4.1
Dressing percentage	63.1	62.2	62.6	62.4	63.0	63.7	0.185	0.4
Marbling score[a]	512.9	534.3	521.9	513.2	521.4	536.4	0.648	12.3
Quality grade[b]	9.6	9.9	9.6	9.5	9.7	9.7	0.631	0.2
Fat thickness, cm	1.1	1.2	1.2	1.1	0.9	1.1	0.748	0.1
Adj. preliminary yield grade	3.3	3.3	3.3	3.3	3.1	3.2	0.755	0.1
Kidney, pelvic, and heart fat, %	1.8	1.9	1.9	2.0	2.0	1.8	0.230	0.1
Longissimus muscle area, cm^2	77.1	77.6	75.1	75.7	77.2	79.2	0.628	1.7
USDA yield grade	3.00	3.03	3.19	3.10	2.85	2.83	0.540	0.15
Lean color score[c]	6.9	6.7	6.7	6.8	6.4	6.8	0.619	0.2
Lean texture score[c]	6.3	6.7	6.6	6.2	6.2	6.3	0.749	0.3
Lean firmness score[c]	6.3	6.7	6.8	6.4	6.4	6.7	0.700	0.3
Skeletal maturity	A^{62}	A^{64}	A^{69}	A^{63}	A^{65}	A^{68}	0.343	2
Lean maturity	A^{58}	A^{63}	A^{64}	A^{63}	A^{67}	A^{64}	0.164	2
Incidence of dark cutters, %	0	0	0	0	0	0	1.000	—
Incidence of heat ring, %	0	0	0	0	0	0	1.000	—
CIE L* value (lean lightness)	38.65	38.96	38.56	39.10	39.01	38.79	0.995	0.74
CIE a* value (lean redness)	19.21	17.32	18.02	18.20	18.20	18.79	0.223	0.53
CIE b* value (lean yellowness)	3.56^{d}	1.51^{f}	1.81^{ef}	2.79^{de}	2.60^{de}	2.82^{de}	0.009	0.39
Saturation index	19.59	17.52	18.23	18.56	18.46	18.91	0.158	0.52
Hue angle	10.48	7.68	7.99	9.06	8.40	9.10	0.651	1.18

[a]Marbling Score: 400 = slight[00], 500 = small[00].
[b]Quality Grade: 9 = USDA Low Choice, 10 = USDA average Choice.
[c]Lean color, texture, firmness: 8 = extremely bright cherry red, fine, firm; 1 = extremely dark, coarse, soft.
[d,e,f]Means in the same row with different superscripts differ ($P < 0.05$).

and 7.5×10^6 IU/steer daily. Cooking decreased the liver vitamin D_3 concentrations by only 10% when steers were treated with 5×10^6 IU/steer daily of VITD, compared to a 27% reduction in controls. Treating cattle with 0.5×10^6 IU/steer daily of vitamin D_3 for 9 d did not result in a significant increase of vitamin D_3 or 25-hydroxyvitamin D_3 residues compared to those in controls in any tissue measured. Treatment effects on concentrations of 1,25-dihydroxyvitamin D_3 in beef (loin) tissue were fairly minimal, but there was a linear decrease ($P < 0.01$) in raw liver concentrations.

Treatment effects on WBS tenderness are presented in Table 6. There was a VITD × muscle × postmortem aging interaction ($P < 0.001$) for WBS values. Strip loin steak WBS was decreased ($P < 0.05$) by treating steers with 0.5 or 7.5×10^6 IU/steer daily at 7 d postmortem compared to controls. Overall, VITD supplementation decreased ($P < 0.01$) strip loin WBS at 7 d postmortem,

Table 4. Effect of feeding vitamin D_3 to steers for nine consecutive days before slaughter on longissimus calcium and phosphorus content and moisture factors, carcass pH, carcass temperature, and calpastatin and calpain activity

Item	Vitamin D_3 treatment, $\times 10^6$ IU/steer daily							
	Control	0.5	1.0	2.5	5.0	7.5	*P*-value	SEM
Calcium content, mg/100 g	48.6^{e}	57.2^{de}	77.9^{c}	65.1^{cd}	65.1^{cd}	76.6^{c}	0.008	5.7
Phosphorus content, mg/100 g	204.7	196.3	199.4	198.1	197.1	193.1	0.753	5.3
Moisture, %	69.99	69.69	69.94	70.09	70.21	69.73	0.893	0.34
Free water, %	9.39	9.07	10.12	10.11	10.10	9.68	0.851	0.68
Bound water, %	90.61	90.93	89.88	89.89	89.90	90.32	0.851	0.68
Immobilized water, %	81.22	81.86	79.76	79.78	79.79	80.64	0.851	1.35
Calpastatin activity[a]	0.55	0.41	0.41	0.32	0.53	0.53	0.818	0.14
μ-Calpain activity[a]	0.50	0.51	0.45	0.38	0.49	0.40	0.767	0.08
m-Calpain activity[a]	0.34	0.37	0.29	0.29	0.38	0.29	0.881	0.07
3-h Carcass temperature, C[b]	34.00	33.50	33.04	32.96	32.67	32.88	0.102	0.34
24-h Carcass temperature, C[b]	6.99^{c}	5.52^{cd}	4.95^{d}	4.19^{d}	5.41^{d}	5.35^{d}	0.033	0.54
3-h Carcass pH[b]	6.13	6.29	6.23	6.13	6.16	6.25	0.675	0.08
24-h Carcass pH[b]	5.49	5.61	5.58	5.58	5.57	5.57	0.821	0.08

[a]Calpain and calpastatin activities were determined on prerigor longissimus samples and represent units of activity per gram.
[b]Carcass temperature and pH were measured between the 11th and 12th ribs of the longissimus thoracis.
[c,d,e]Means in the same row with different superscripts differ ($P < 0.05$).

Table 5. Effect of feeding vitamin D_3 to steers for nine consecutive days before slaughter on vitamin D_3 concentrations and two of its metabolites, 25-hydroxyvitamin D_3 (25-OH-Vitamin D_3) and 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2$-Vitamin D_3], in strip loin steaks, liver, and cooked liver samples

	Vitamin D_3 treatment, $\times 10^6$ IU/steer daily							
Item	Control	0.5	1.0	2.5	5.0	7.5	*P*-value	SEM
Strip loin steaks[a]								
Vitamin D_3, ng/g	9.5^{b}	18.3^{bc}	23.7^{cd}	37.3^{d}	75.2^{e}	65.9^{e}	< 0.001	3.9
25-OH-Vitamin D_3, ng/g	4.1^{b}	5.6^{b}	6.6^{b}	6.5^{b}	11.5^{c}	12.4^{c}	< 0.001	1.0
1,25-$(OH)_2$-Vitamin D_3, pg/g	202.7	229.2	130.0	143.0	171.1	172.3	0.305	32.8
Liver[a]								
Vitamin D_3, ng/g	140.8^{b}	256.1^{bc}	365.5^{cd}	451.9^{de}	563.5^{de}	508.4^{de}	< 0.001	60.7
25-OH-Vitamin D_3, ng/g	1.9^{b}	5.7^{bc}	6.7^{bc}	7.5^{bc}	13.2^{d}	11.3^{cd}	0.020	2.3
1,25-$(OH)_2$-Vitamin D_3, pg/g	259.4	212.7	93.2	71.9	15.1	81.8	0.066	60.9
Cooked liver (75°C)[a]								
Vitamin D_3, ng/g	103.5^{b}	197.6^{b}	302.3^{bc}	325.7^{cd}	509.9^{cd}	451.6^{cd}	0.010	70.9
25-OH-Vitamin D_3, ng/g	2.2^{b}	5.5^{b}	4.3^{b}	6.2^{b}	20.0^{c}	14.9^{c}	0.003	2.9

[a]Tissue sample concentrations are expressed on a wet or fresh tissue basis.
[b,c,d,e]Means in the same row with different superscripts differ ($P < 0.05$).

but WBS on postmortem d 10, 14, and 21 were not affected ($P > 0.05$) by treatment (Table 2). Thus, VITD treatment accelerates the aging process by shifting calcium to the muscle cell. There was a quadratic treatment effect on round steak WBS as a result of treatment at d 7 postmortem ($P < 0.01$) and at d 10 postmortem ($P < 0.05$). Treating steers with 0.5, 1, and 5×10^6 IU/steer daily of VITD decreased 7-d postmortem round WBS ($P < 0.05$) compared with controls (Table 6). All VITD treatments lowered round steak WBS at 10 d postmortem ($P < 0.05$). There was no treatment effect on 10-d postmortem round steak WBS, but 0.5×10^6 IU/steer daily lowered ($P < 0.05$) round steak WBS at 21 d postmortem. Vitamin D_3 supplementation effects on strip loin WBS were fairly minimized, indicated by low shear force values. Vitamin D_3 treatment had its greatest impact on improving WBS tenderness in round steaks.

Strip loin and round steaks were aged to 7 d postmortem only for sensory panel determinations. There was a significant ($P = 0.015$) vitamin D_3 treatment effect on initial juiciness scores. Vitamin D_3 treatment decreased ($P < 0.05$; Table 7) initial juiciness scores of strip loin steaks when steers were supplemented with 5×10^6 IU/steer daily, and round steak initial juiciness was increased ($P < 0.05$) when steers were treated with 1×10^6 IU/steer daily of VITD. Sustained juiciness, beef flavor, and off-flavors were not affected ($P > 0.05$) by treatment in either muscle. A muscle × vitamin D_3 interaction affected initial ($P < 0.001$) and sustained tenderness ($P = 0.005$) and beef flavor intensity ($P = 0.007$) scores. Strip loin steak initial and sustained tenderness scores from treated steers did not differ from those of controls ($P > 0.05$); however,treating cattle with 0.5, 1, 5, or 7.5×10^6 IU/steer daily of VITD increased ($P < 0.05$) round initial tenderness scores, and all VITD

Table 6. Effect of feeding vitamin D_3 to steers for nine consecutive days before slaughter on Warner-Bratzler shear force of strip loin and top round steaks aged to 7, 10, 14, or 21 d postmortem

	Vitamin D_3 treatment × 10^6 IU/steer daily					
Days postmortem	Control	0.5	1.0	2.5	5.0	7.5
	Strip loin steaks					
7 d	2.80^{abcde}	2.22^{g}	2.53^{defg}	2.58^{cdefg}	2.47^{defg}	2.31^{fg}
10 d	2.66^{abcdef}	2.56^{cdefg}	2.64^{bcdef}	2.81^{abcde}	2.74^{abcde}	2.78^{abcde}
14 d	2.92^{abc}	2.79^{abcde}	2.81^{abcde}	3.01^{a}	2.84^{abcd}	2.97^{ab}
21 d	2.45^{efg}	2.56^{cdefg}	2.48^{defg}	2.67^{abcde}	2.52^{defg}	2.57^{cdefg}
	Top round steaks					
7 d	4.50^{a}	3.67^{fghijk}	3.52^{jk}	4.29^{abc}	3.63^{ghijk}	4.38^{ab}
10 d	4.39^{ab}	3.65^{ghijk}	3.48^{k}	3.81^{efghijk}	3.86^{efghij}	3.57^{ijk}
14 d	4.01^{cdefg}	4.40^{ab}	3.95^{cdefgh}	4.27^{abc}	4.25^{abcd}	4.14^{abcde}
21 d	4.16^{abcde}	3.59^{hijk}	3.80^{efghijk}	4.14^{abcde}	4.04^{bcdef}	3.89^{defghi}

[a,b,c,d,e,f,g,h,i,j,k]Means for each muscle with different superscripts differ ($P < 0.05$). Means with different letters differ between days and treatments for each of the two steak types. SEM = 0.13; vitamin D_3 × muscle × postmortem age interaction *P*-value was < 0.002. Each value is the mean of seven pens (28 steers).

Table 7. Effect of feeding vitamin D_3 to steers for nine consecutive days before slaughter on sensory panel traits of top loin and top round steaks aged to postmortem d 7

Item	Vitamin D_3 treatment, $\times 10^6$ IU/steer daily						
	Control	0.5	1.0	2.5	5.0	7.5	SEM
	Strip loin steaks						
Initial juiciness[a]	5.98[f]	5.74[fg]	5.91[f]	5.90[f]	5.35[g]	5.88[f]	0.14
Sustained juiciness[a]	6.13	5.96	6.07	6.07	5.69	6.10	0.14
Initial tenderness[b]	6.75	6.47	6.65	6.42	6.45	6.42	0.18
Sustained tenderness[b]	6.91[fg]	6.82[fg]	6.99[f]	6.39[g]	6.78[fg]	6.64[fg]	0.20
Flavor intensity[c]	6.50[fg]	6.36[gh]	6.40[g]	6.28[gh]	6.17[h]	6.65[f]	0.08
Beef flavor[d]	6.61	6.63	6.78	6.42	6.54	6.57	0.10
Off-flavor[e]	1.12	1.08	1.03	1.11	1.08	1.08	0.05
	Top round steaks						
Initial juiciness[a]	5.31[g]	5.58[fg]	5.92[f]	5.64[fg]	5.35[g]	5.40[g]	0.14
Sustained juiciness[a]	5.65	5.89	6.15	6.00	5.81	5.58	0.14
Initial tenderness[b]	4.26[h]	5.21[fg]	5.58[f]	4.72[gh]	5.61[f]	5.05[fg]	0.18
Sustained tenderness[b]	4.37[h]	5.46[fgh]	5.70[fg]	5.03[h]	5.81[f]	5.20[gh]	0.20
Flavor intensity[c]	6.12[g]	6.21[fg]	6.40[f]	6.33[fg]	6.17[fg]	6.19[fg]	0.08
Beef flavor[d]	6.26	6.45	6.58	6.46	6.44	6.12	0.10
Off-flavor[e]	1.19	1.15	1.28	1.17	1.15	1.29	0.05

[a]Juiciness: 6 = moderately juicy, 7 = very juicy.
[b]Tenderness: 5 = slightly tender, 6 = moderately tender.
[c]Flavor intensity: 6 = moderately intense flavor, 7 = very intense flavor.
[d]Beef flavor: 6 = moderately characteristic beef flavor, 7 = very characteristic beef flavor.
[e]Off-flavor: 1 = none, 5 = extremely off-flavor.
[f,g,h]Means for each muscle with different superscripts differ ($P < 0.05$).

treatments increased ($P < 0.05$) sustained tenderness scores for round steaks compared with controls. Sensory panel scores indicated that VITD treatment had its greatest improvement in tenderness scores in round steaks. Beef flavor intensity was decreased ($P < 0.05$) in strip steaks by supplementing cattle with 5×10^6 IU/steer daily, whereas supplementing with 1×10^6 IU/steer daily resulted in increased ($P < 0.05$) beef flavor intensity in round steaks. Treatment effects on juiciness and flavor tended to be minor or insignificant.

Discussion

Vitamin D_3 has long been known to have an essential role in vertebrates. Vitamin D_3 helps mediate calcium and phosphorus metabolism to target tissues including, intestine, bone, kidney, and even muscle (de Boland and Nemere, 1992). Specifically, vitamin D_3 is classified as a secosteroid and requires conversion to its steroid form to have full biological activity (DeLuca, 1979). Vitamin D_3 from dietary sources is taken up by the bloodstream in the intestine or is produced within the body by ultraviolet light conversion of 7-dehydrocholesterol. Vitamin D_3 is then hydroxylated within the liver to 25-hydroxyvitamin D_3. Further processing occurs in the kidneys, where 25-hydroxyvitamin D_3-1α-hydroxylase introduces a hydroxyl group at the α-position of carbon 1 of the A ring, producing the active steroid 1,25-dihyroxyvitamin D_3 (Reichel et al., 1989). This final reaction yields an active metabolite that has biological activity 500- to 1,000-fold higher than its precursor 25-hydroxyvitamin D_3. Then, 1,25-dihyroxyvitamin D_3 plays a vital role in regulating calcium and phosphorous homeostasis by forming a steroid-receptor complex in target cells, to initiate the synthesis of specific RNA-encoding proteins that mediate calcium-binding responses (Wecksler and Norman, 1980; Pike, 1985; Costa et al., 1986).

Feeding high doses of VITD can cause vitamin D_3 toxicity in cattle, depending on the dose and length of feeding, resulting in prolonged hypercalcemia, weight loss, loss of appetite, decreased feed intake, and death (Littledike and Horst, 1982; Mortensen et al., 1993). Puls (1994) suggested that supplementing cattle with 1 to 2×10^6 IU of VITD/animal daily could result in toxicity. Therefore, the doses used in this experiment could pose potential vitamin D_3 toxicity concerns. In our experiment, concentrations of 2.5×10^6 IU/steer daily of VITD and higher negatively affected feedlot performance, whereas 1×10^6 IU/steer daily of VITD or less did not have this negative effect. Feeding VITD at higher doses or longer periods of time than in the present experiment would presumably have a negative impact on feedlot performance.

Our experiment confirmed the work of Swanek et al. (1999) and Montgomery et al. (2000), indicating that VITD supplementation improves beef tenderness of the strip loin and top round. This is the first experiment to indicate VITD supplementation as low as 0.5×10^6 IU/animal daily improved beef tenderness. Furthermore, the strip loin steaks in our experiment were generally tender, averaging WBS value of less than 3 kg.

The most significant influence on beef tenderness that VITD supplementation had in our study was on the generally tougher round steaks. Thus, VITD feeding should effectively improve beef tenderness when cattle tend to be tough and have no or little impact on cattle that produce tender beef. Injection of carcasses and meat cuts with calcium chloride ($CaCl_2$) has been shown to improve steak tenderness (Wheeler et al., 1993; Kerth et al., 1995; Lansdell et al., 1995). Vitamin D_3 supplementation seems to accelerate postmortem aging similarly to infusing carcasses with $CaCl_2$ solutions shortly after death (Koohmaraie et al., 1988, 1989, 1990).

Both Swanek et al. (1999) and Montgomery et al. (2000) indicated that VITD treatments accelerated postmortem tenderization of beef; lowered WBS appeared at d 14 but disappeared by d 21 postmortem. Similarly, in our study the improvements in WBS diminished by 21 d postmortem. The link between postmortem aging, myofibrillar degradation, and calcium has long been known (Davey and Gilbert, 1969; Busch et al., 1972). The effect of postmortem tenderization of beef seems to be the result of degradation of key myofibrillar proteins. The calcium-activated protease μ-calpain has been shown to proteolectically degrade five key skeletal muscle proteins, titin, nebulin, filamin, desmin, and troponin T, during postmortem storage (Huff-Lonergan et al., 1996a,b). The VITD improvement of tenderness in our experiment was probably a result of this increased proteolysis, which has been previously shown in VITD-supplemented cattle (Montgomery et al., 2000).

Swanek et al. (1999) indicated that VITD supplementation increased water-extractable muscle calcium and lowered calpain activity. Our experiment showed that VITD supplementation increased plasma and total muscle calcium, yet calpain and calpastatin acivities were not affected. The difference between these two studies in calpain activity might be attributable to sample collection times. We sampled carcasses at 20 min postmortem, whereas Swanek et al. (1999) sampled at 24 h postmortem. During the early stages of rigor mortis muscle creatine phosphate is used to convert ADP to ATP. When these reservers are depleted, the ATP level in the muscle falls and anerobic glycolysis in the muscle produces lactate from glycogen, resulting in a decrease in muscle pH. During the postmortem period, the pH decreased from 7.2 to 5.4 in a short peroid of time (Penny, 1980). This decrease in pH weakens the myosin-actin cross-bridges slightly, releasing bound calcium (Palmer and Kentish, 1994). The lowering of muscle pH also causes a decrease in protein binding affinity for calcium ions, increasing free cytosolic Ca^{2+} concentrations (El-Saleh and Solaro, 1988; Gulati and Babu, 1989; Parsons et al., 1997). Cytoplasmic concentrations of free Ca^{2+} in skeletal muscle range from 0.05 to 0.10 μ*M* at rest to 10 to 20 μ*M* during an action potential (Konishi, 1998). Because of the drop in pH due to the buildup of lactate there is a large increase in free skeletal muscle calcium during the first 24 h postmortem (Khan and Kim, 1975) when muscle is converted to meat. Because the calcium requirement for half-maximal activity of μ-calpain is 3 to 50 μ*M* and for m-calpain is 200 to 1,000 μ*M* of free Ca^{2+} in skeletal muscle (Barrett et al., 1991; Goll et al., 1992), any increase in free calcium during the first 24 h postmortem can lead to large changes in calpastatin and calpain activity (Boehm et al., 1998). Swanek et al. (1999) found that VITD treatment increased free calcium from 350 μ*M* to 530 μ*M*, resulting in a lowering of calpain activity. We did not find an effect of VITD treatment on calpain activity, presumably because of early sampling; however, VITD treatment accelerated postmortem aging of steaks. This effect was probably a result of elevated muscle calcium concentrations and increased proteolysis during postmortem aging.

The recommended dietary allowances of VITD for an adult human is 200 IU/d and is 400 IU/d for young adults, which is 5 and 10 μg, respectively (NRC, 1989). At this rate, an adult would need to eat 67 g of steak or 9 g of liver from steers treated with 5×10^6 IU of VITD to meet his or her daily needs for this nutrient. Consumption of as little as 45 μg of VITD per day has been associtaed with signs of vitamin D_3 toxicity in young children (American Academy of Pediatrics, 1963). At this rate, it would take as little as 88 g of cooked liver from treated cattle to deliver a potential toxic dose of VITD. A more recent study found that the minimum dose required to cause toxicity was 1,250 μg of VITD, which would require of 16 kg of beef or 2.2 kg of liver to receive a toxic dose (Miller and Hayes, 1982). Olson et al. (1972) first studied the effects of administering high amounts of 25-hydroxyvitamin D_3 to cattle and found a 140% increase in VITD concentrations in beef. Our experiment and the one conducted by Montgomery et al. (2000) indicate that feeding VITD in excess of 0.5×10^6 IU/animal daily significantly raised VITD concentrations in beef and liver. Cooking samples also decreased the concentrations of residues. The increase in residues in liver samples poses a serious toxicological hazard, requiring livers to be removed from the food chain. However, the increase in beef muscle VITD concentrations does not seem to pose a toxicological hazard. Assessment of toxicological hazards in tissues from VITD-treated cattle is further complicated by results suggesting that the dietary allowances are grossly underestimated and should be increased to at least 250 μg/d for adults (Vieth, 1999). Because of the complications associated with calculating toxicological hazards associated with VITD, cattle should be supplemented at a level such as 0.5×10^6 IU/animal daily to prevent a significant increase in tissue residues.

The 1995 National Beef Quality Audit listed tenderness as a major beef quality problem and the second-largest concern of the retail industry (Smith et al., 1995). This experiment confirmed that short-term feeding of VITD improved beef tenderness because of increased muscle calcium. The increased muscle calcium

probably led to increased proteolysis during early postmortem storage, accelerating postmortem tenderization. Future studies should further investigate the lower VITD treatments used here to determine influences on beef tenderness in a variety of muscles. Moreover, VITD supplementation effects on cattle of different biological types still require further investigation. In summary, feeding VITD at a level of 0.5×10^6 IU/animal daily is a simple, short-term, cost-effective means to improve beef tenderness without negatively affecting feedlot performance, carcass traits, or tissue residues.

Implications

Feeding vitamin D_3 to increase muscle calcium seems to be a cost-effective means of improving beef tenderness. Supplementing steers with levels of 1×10^6 IU/animal daily of vitamin D_3 and greater negatively affected feedlot performance and increased tissue residues. This experiment indicated that feeding vitamin D_3 at a dose of 0.5×10^6 IU/animal daily of vitamin D_3 improved beef tenderness without negatively affecting feedlot performance or tissue residues. Future studies should be conducted to investigate the effect of biological type and sex on the effectiveness of vitamin D_3 improvement of beef tenderness in a variety of beef muscles.

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